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PRINCIPAL INVESTIGATOR: Fen Wang, Ph.D

CONTRACTING ORGANIZATION: Texas A&M University System Health
Science Research Foundation
College Station, Texas 77843-3578

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13. ABSTRACT (Maximum 200 Words) Purpose: To study the FGF signaling axis in prostate homeostasis and tumorigenesis, to evaluate dietary factors in modulating FGF signals in the prostate. Scope: to develop mouse models resembling human prostate tumor progressions for screening therapeutic strategies for prostate cancers and evaluating dietary factors in prostate cancer prevention. Major Finding: Ectopic expression of the constitutively-active GFGR1 (caGFGR1) in the prostate induces high-grade prostatic intraepithelial neoplasia (PIN) in transgenic mice. The development of PIN and degree of homeostasis perturbation in the prostate are caGFGR1 expression level-dependent. Repression of the resident FGFR2 in the prostate poentiated the lesions induced by the ectopic caGFGR1. Up-to-date Progress: The major findings are published in Cancer Research. We have established mouse colonies with prostate-specific disruption of Fgfr2 loci and expression of the ectopic caGFGR1 for further characterizations of the FGF signaling and dietary factors in prostate lesions. Significance: Together with previous data form the Dunning prostate tumor model, the findings demonstrate that aberrant FGF signals in the prostate strongly disrupt tissue homeostasis, and promote prostate tumor development and progression. The model provides a useful tool for evaluating other tumor initiating factors, including those that cause genetic instability and other oncogenic lesions in prostate tumorigenesis.				
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INTRODUCTION

The prostate consists of epithelium and stromal compartments separated by a basement membrane. The communications and mutual regulations between the two compartments are critical for growth, develop, and function of the prostate. The fibroblast growth factor (FGF) signaling complex has long been implicated in mediating these regulatory communications, which consists of 23 single polypeptide ligands, 4 transmembrane tyrosine kinase receptors (FGFR), and a pool of highly heterogenic heparan sulfate proteoglycans (HSPG) in the extracellular matrix. Appearances of the FGF signaling complexes are highly temporally and spatially specific; aberrant expression is often correlated with prostate cancer progression in human and experimental animal models. We previously demonstrated that ectopic expression of a constitutively active FGFR1 mutant (caFGFR1) in the prostate epithelium induces age- and expression level-dependent prostatic intraepithelial neoplasia (PIN). Furthermore, depression of FGFR2 signaling in the prostate also disturbs homeostasis in the prostate and induces prostate hyperplasia. The project is to study whether FGFR1 and FGFR2 elicit receptor-specific activity in the prostate, to determine whether ectopic expression of FGFR1 and depression of FGFR2 synergistically induce prostatic lesion, and to evaluate dietary factors in modulating FGF signals in the prostate. The long-term goal is to develop mouse models resembling human prostate tumor progressions for screening therapeutic strategies for prostate cancers and evaluating dietary factors in prostate cancer prevention.

BODY

Task 1. Characterization of the prostate of caFGFR1/KDNR bigenic mice.

- a. *Generation of enough male caFGFR1/KDNR bigenic mice for the study by crossing caFGFR1 and KDNR transgenic mice.*
- b. *Collecting prostate tissue of bigenic and control mice at different ages, performing serial section of the prostate and characterization of prostate tissue structures.*

Progress:

1. Characterization of the ARR2PBi-caFGFR1/KDNR bigenic mice.

We previously generated PB-caFGFR1 transgenic mice that expressed low levels of caFGFR1 induced age-dependent progressive PIN accompanied by stroma thickening (Wang *et al.*, 2004). To determine whether expression of the caFGFR1 kinase at high levels induced more profound phenotypes in younger mice, we generated the second generation of caFGFR1 transgenic mice, the ARR2PBi-caFGFR1, with the improved

ARR2PB composite promoter to direct expression of the caFGFR1 in prostate epithelium. Both RT-PCR and RNase protection assay (RPA) clearly showed that the ARR2PBi-caFGFR1 was expressed only in the prostate of transgenic mice (Jin *et al.*, 2003a). The ARR2PBi-caFGFR1 mice were then crossed with the KDNR mice that had prostate-specific depression of FGFR2 signaling axis as reported (Foster *et al.*, 2002), for generation of the ARR2PBi-caFGFR1/KDNR bigenic mice. We have generated the bigenic colony and the two parental mice (ARR2PBi-caFGFR1 and KDNR), as well as the wildtype littermates for characterization of FGF signaling in the prostate and for evaluation of dietary factors in the prostate. To date, the prostates of bigenic mice and the parental transgenic strains have been phenotypically characterized, and the main results are published in Cancer Research (Jin *et al.*, 2003a). The outlines of the main findings are as follows; please refer to the attachments for the details.

2. ARR2PBi-caFGFR1 mice developed high grade PIN within 8 months.

In general, ARR2PBi-caFGFR1 mice exhibited no significant phenotype and were reproductively active before the age of 3-4 months, which were similar to the PB-caFGFR1 mice that expressed caFGFR1 kinase at low levels in the prostate. The average wet tissue weight of the ARR2PBi-caFGFR1 prostate was 131 ± 20 mg ($n=13$), and the average prostate tissue weight of WT littermates was 78 ± 14 mg ($n=30$). To study the time-dependent developments of prostatic lesions induced by the caFGFR1 kinase at high dosages, prostate tissues were collected from ARR2PBi-caFGFR1 mice and WT littermates at the ages of 3 to 12 months old. The prostate tissues were completely sectioned; one out of every five slides was selected, stained with hematoxylin and eosin, and examined histologically. No significant difference between the prostate tissues of ARR2PBi-caFGFR1 mice and WT littermates at the age of 3-4 months was observed. However, most ARR2PBi-caFGFR1 prostates developed PIN 3 to PIN 4 lesions in every lobe when the mice were older than 8 months. In the foci with high grade PIN, the lumens were filled with disorganized, atypical epithelial cells. The nuclear to cytoplasmic ratio was significantly increased. Hyperchromatic nuclei with clearly visible nucleoli were present. Areas of cells with a cribriform pattern were observed in both dorsal-lateral and anterior lobes. Frequently, epithelial cells were observed pushing into the stromal layers and formed microinvasion foci in the stromal layer. Mitotic figures were also apparent in the foci with high grade PIN. Cellular and nuclear atypia were clearly more pronounced compared to that of PB-caFGFR1 prostates that only expressed the caFGFR1 at low-levels and developed intermediate PIN at similar ages.

3. Histochemical characterization of the prostate of ARR2PBi-caFGFR1 mice.

To determine whether cells within the PIN foci in the ARR2PBi-caFGFR1 prostate expressed cytokeratins that are characteristic markers of well-differentiated prostate epithelial cells, the prostate sections of the ARR2PBi-caFGFR1 and WT littermates were stained with anti-pan cytokeratins. The results clearly revealed that the expression of cytokeratins was significantly reduced in epithelial foci with high-grade PIN lesions,

especially those with cribriform structures. Whereas the cells in relatively normal areas, even those adjacent to the PIN foci, still highly expressed cytokeratins as the cells in WT prostates. In addition, the high-grade PIN foci, particularly those with cribriform structures, often had a disrupted stromal layer indicated by discontinuous and faint staining of smooth muscle cell characteristic α -actin. In contrast, the epithelial compartment of WT prostates was usually surrounded by well-organized stromal cells. Immunostaining with anti proliferating cell nuclear antigen (PCNA) revealed that the cells in the prostate of RR2PBi-caFGFR1, but not wildtype littermates, were actively engaged in proliferation. PAS staining, which stained polysaccharides in the basement membrane that separated the epithelial and stromal compartments, revealed that the basement membrane in the ARR2PBi-caFGFR1 prostate was discontinuous, especially around the high-grade PIN foci. Frequently, the over proliferating epithelial cells disrupted the basement membrane, invaded the stromal compartment, and formed small epithelial foci in the space between the basement membrane and stromal cells. This indicated that the epithelial cells in these foci were not just pushed through the stromal layer and formed glandule-like structures. Instead, these epithelial cells formed microinvasion foci in the stromal compartment, which were more advanced lesions than high grade PIN.

4. Cooperation between ectopic expression of FGFR1 and depression of FGFR2 signaling in perturbation of prostate homeostasis.

To determine whether disruption of the endogenous FGFR2 signaling axis and ectopic appearance of FGFR1 in prostate epithelial cells had synergistic effects in disturbing prostatic homeostasis, we generated ARR2PBi-caFGFR1/KDNR bigenic mice by crossing. Like the parental strains, young adult bigenic mice did not exhibit significant abnormality in the prostate. Yet, the bigenic prostate gradually developed high-grade PIN foci when the mice were older than 6 months, which was similar to the mice only expressing high levels of caFGFR1. To determine whether ectopically expressed FGFR1 kinase further increased the neuroendocrine (NE) cell population, tissue sections of prostates from the ARR2PBi-caFGFR1/KDNR, ARR2PBi-caFGFR1, KDNR, and WT mice were immunohistochemically stained with anti-synaptophysin antibody. The results clearly demonstrated that the NE cell population in the bigenic mice was significantly higher than in both KDNR and ARR2PBi-caFGFR1 counterparts. As reported earlier, the KDNR prostate had an increased NE cell population in the prostate (Foster *et al.*, 2002). In addition, the NE cell population in the ARR2PBi-caFGFR1 prostate was also somewhat increased compared with that of WT littermates.

5. Depression of the FGFR2 signaling potentiates PIN lesion development induced by low-level caFGFR1.

To determine whether repression of FGFR2 signaling promoted PIN lesion development in PB-caFGFR1 mice that only expressed caFGFR1 kinase at low levels in the prostate, we then generated PB-caFGFR1/KDNR bigenic mice by crossing. The

prostate tissues of the bigenic and both monogenic parental strains were collected for pathological examination at different ages. The PB-caFGFR1 and KDNR parental strains usually developed high-grade PIN foci only when the mice were older than 18 months. In contrast, the prostate of PB-caFGFR1/KDNR bigenic mice frequently developed high-grade PIN within 8 months, which was significantly earlier than both parental strains. Most of the lumen of the gland was filled with multiple layers of atypical epithelial cells with hyperchromatic nuclei and prominent nucleoli. Cellular and nuclear atypia was clearly more prominent compared to the intermediate PIN in the PB-caFGFR1 and the KDNR mice at similar ages. The nuclear to cytoplasmic ratio was significantly increased in the cells within the PIN foci, as well. Furthermore, the NE cell population in the PB-caFGFR1/KDNR bigenic prostate also significantly increased. PAS staining indicated disruption of the basement membrane and microinvasion of epithelial foci in the stromal compartment. Trichrome staining also revealed that hyperplastic stroma did not exhibit excessive collagen staining in either ARR2PBi-caFGFR1/KDNR or PB-caFGFR1/KDNR bigenic mice, as reported earlier.

Task 2. Generation of ROSA-caFGFR1/Fgfr2^{c/c} mice, and characterization of the prostates.

- a. Construction of the ROSA-caFGFR1 knock-in vector.
- b. Introduction of the ROSA-caFGFR1 construct to mouse embryo stem cells.
- c. Generation of founders bearing the ROSA-caFGFR1 locus.
- d. Establishing colonies from each positive founder and verifying germ line integration of the ROSA-caFGFR1 locus.
- e. Collecting prostates from ROSA-caFGFR1/Fgfr2^{c/c} and control mice, performing serial section of the prostate and characterization of the prostate tissue structures.
- f. Assess the impact of dietary factors on the initiation and development of prostate lesions in ROSA-caFGFR1/Fgfr2^{c/c} mice.

Progress:

1. Construction of the ROSA-caFGFR1 knock-in vector.

The ROSA26 locus encodes an unessential gene that is strongly and ubiquitously expressed, which is ideal for tissue specific expression of a target gene together with the Cre-loxP recombination technology. We had constructed the ROSA26 targeting vector that consisted of exon 1 and the adjacent intronic sequences, exon 3 and the adjacent intronic sequences of ROSA26 locus for homologue recombination, a mRNA splice acceptor site followed by a loxP flanked neo cassette that contained multiple stop

codons and multiple polyA additional sites. The vector had been proven to successfully knock-in several target sequences in the ROSA26 locus. To knock-in caFGFR1 cDNA in the ROSA locus, the caFGFR1 cDNA together with an SV40 RNA splice site and a polyA addition site was inserted in the multiple cloning sites between the loxP flanked neo cassette and exon 3 of ROSA26 as depicted in Fig. 1. The DNA fragment was cloned in the pGL3 vectors. Since the caFGFR1 cDNA is inserted downstream of the neo cassette which has multiple translational stop sites and polyA additions sites, the caFGFR1 cDNA will not be transcribed until the loxP flanked neo cassette is removed by Cre recombinase.

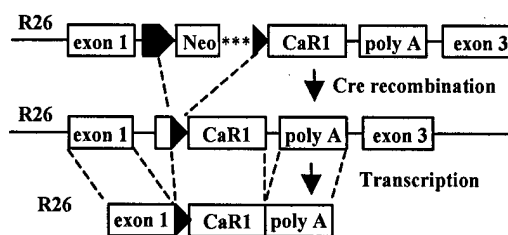


Fig. 1. Scheme for generation of ROSA-caFGFR1 mice. When the loxP flanked neo cassette is deleted by Cre recombinase, the caFGFR1 transgene will be transcribed. After splice modification, the matured transcript only encodes caFGFR1. Triangles, loxP sites; hatch box, splice acceptor sequence; neo, neo cassette; *, translational stop codon; caFGFR1, caFGFR1 cDNA; polyA, the splice site and the polyA addition site of SV40 T antigens; exon1, 3, exon1, 3 of the ROSA26 locus.

2. Introduction of the ROSA-caFGFR1 targeting vector to mouse embryonic stem cells and generation of ROSA-caFGFR1 mice.

The ROSA-caFGFR1 DNA was excised from the vector and introduced to AK7 mouse embryo stem (ES) cells by electroporation. The transfected AK7 cells were selectively grown in the presence of geneticin. About two hundred geneticin resistant clones were selected for Southern analyses with the ROSA probe. Five colonies were identified to have correct recombination. Among them, #76 was selected for the microinjection. The microinjected blastocysts were transferred to a pseudopregnant foster mother for full term development. Four chimera founders were generated from the injection. Currently, at least one founder has germ line integration, as demonstrated with the PCR screen of caFGFR1 in the agouti offspring (Fig. 2). Southern analysis with the ROSA probe will be used to confirm the PCR results soon. Currently, we are in the process of establishing the colonies.

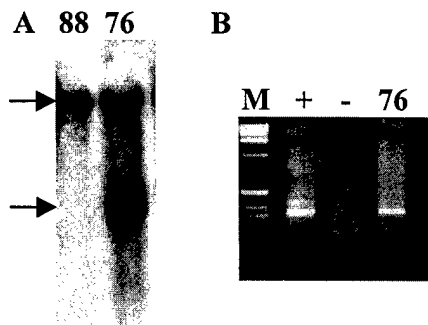


Fig. 2. A. Integration of caFGFR1 cDNA in the ROSA locus. The indicated ES clones selected from the geneticin resistant cultures were southern analyzed with the ROSA probe. The upper band represents the intact locus, and the bottom band represents the caFGFR1 knock-in locus. **B. PCR analysis of the F1 agouti offspring from the chimera.** The tail DNA was purified from the agouti pups of the chimera, and analyzed with the RT-PCR as described (Jin *et al.*, 2003a). The 350 base pair band representing the caFGFR1 cDNA was detectable in the agouti offspring of #76. M, 1 kb molecular weight marker; +, positive control; - negative control; 76, clone 76.

3. Specific activation of caFGFR1 expression and inactivation of Fgfr2 gene in the prostate of ROSA-caFGFR1/Fgfr2^{flx/flx} mice

The ROSA-caFGFR1 mice will then be crossed with the Fgfr2^{flx/flx}-ARR2PBi-Cre mice that have been established recently. The Fgfr2^{flx/flx}-ARR2PBi-Cre mice have a significantly reduced FGFR2 expression in the prostate (Fig. 3). The characterization of the ARR2PBi-Cre mouse was partially supported by this project and was published in *The Prostate* in 2003 (Jin *et al.*, 2003b). The Cre transgenic line will also be used to specifically activate expression of the caFGFR1 target gene in the prostate that is otherwise silent due to the presence of the loxP flanked neo cassette. Once we have established the #76 ROSA-caFGFR1 colonies, we will cross the mice with Fgfr2^{flx/flx} mice to generate ROSA-cafgfr1^{+/+}/Fgfr2^{flx/flx} mice. Since ROSA-cafgfr1 DNA will remain silent and the floxed Fgfr2 will be expressed normally, we do not anticipate the ROSA-caFGFR1^{+/+}/Fgfr2^{flx/flx} mice to exhibit any abnormality. To minimize unnecessary breeding, we have generated female mice with a genotype of Fgfr2^{flx/flx}/ARR2PBi-Cre^{+/-} in advance. The female ARR2PBi-Cre mouse does not express the Cre recombinase; the Fgfr2^{flx/flx}/ARR2PBi-Cre^{+/-} females do not exhibit defects in reproduction as expected. By crossing male ROSA-caFGFR1^{+/+}/Fgfr2^{flx/flx} and female Fgfr2^{flx/flx}/ARR2PBi-Cre^{+/-} mice, 50% of the male offspring will have a desired genotype of ROSA-caFGFR1^{+/+}/Fgfr2^{flx/flx}/ARR2PBi-Cre^{+/-} that has a prostate specific expression of caFGFR1 and prostate specific knock-out of the Fgfr2 gene (ROSA-caFGFR1/Fgfr2^{c/c}). As control animals, we will also breed the Fgfr2^{flx/flx}/ARR2PBi-Cre^{+/-} mice that have a prostate specific knock-out of the Fgfr2 gene (Fgfr2^{c/c}), the ROSA-caFGFR1^{+/+}/ARR2PBi-Cre^{+/-} mice that have a prostate specific expression of caFGFR1, or the ROSA-caFGFR1^{+/+}/Fgfr2^{flx/flx} mice that represents the wildtype mice (WT). Once the male mice reach the age of 4 weeks old, the Cre recombinase will be expressed in the prostate of the mice that carry the ARR2PBi-Cre transgene, which will then excise the loxP flanked sequence in the ROSA-caFGFR1 locus, and the Fgfr2 loci to activate caFGFR1 expression and inactivate FGFR2, respectively.

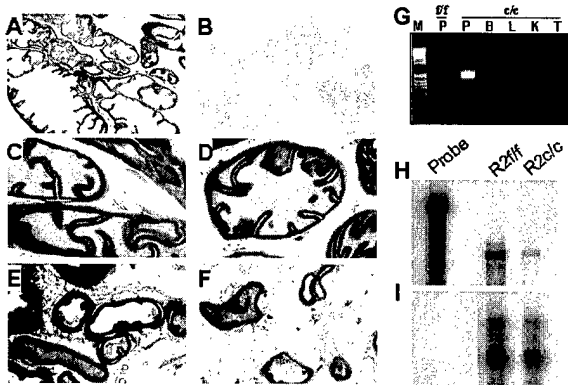


Fig. 3. Expression the Cre recombinase in the ARR2PBi-Cre prostate. A-F, alkaline phosphatase staining. The frozen sections prostate tissues from the ARR2PBi-Cre-Z/AP bigenic mouse (A, C-F), and Z/AP male (B) were staining with the BM-Purple as described. (G). Excision of the loxP flanked Fgfr2 sequence in Fgfr2^{flx}-ARR2PBi-Cre bigenic mice. Genomic DNAs from the prostate and indicated tissues of 10-week old Fgfr2^{flx} (f/f) and Fgfr2^{flx}-ARR2PBi-Cre (c/c) males were extracted. The 500 bp fragment representing condition deleted FGFR2 was PCR amplified as described (Jin *et al.*, 2003b). Note that the 500 bp fragment was only detectable in Fgfr2^{flx}-ARR2PBi-Cre bigenic mice. M, 1 kb DNA marker; P, prostate; B, bladder; L, Liver; K, kidney; T, testis. (H-I). Reduction of FGFR2 expression in Fgfr2^{flx}-ARR2PBi-Cre mice. The total RNAs were extracted from the prostate of Fgfr2^{flx}-ARR2PBi-Cre (R2c/c) and Fgfr2^{flx} (R2f/f) mice. The expression of FGFR2 was accessed by the RNase protection assays with the FGFR2 probe (H), or the β-actin probe for loading controls (I).

4. Characterization of the prostates of the ROSA-caFGFR1/*Fgfr2*^{flx/flx} mice and to evaluation of the effects of dietary factors in prostate lesions induced by the aberrant FGF signaling.

The FGFR2 prostate specific knock-out colonies have been established, and will be characterized in the next few months. Since the age plays an important role in prostate lesions, the impact of aging on the lesion development will be studied. Once we have generated enough mice for experiments, we will carry out pathological studies, including tissue structures, immunohistochemistry analysis. The expression of caFGFR1 and resident FGFR2 will also be accessed. The effects of dietary factors on the FGF signaling axis will also be studied as soon as the colonies are established.

KEY RESEARCH ACCOMPLISHMENTS

1. Establish a highly efficient prostate specific Cre mouse model for tissue specific disruption and activation of genes in the prostate. The Cre recombinase efficiently excises the loxP-flanked fragment in the FGFR2 locus in the prostate.
2. Characterize the phenotype of the ARR2PBi-caFGFR1 transgenic mice that express caFGFR1 at high levels.
3. Characterize the phenotype of the ARR2PBi-caFGFR1/KDNR bigenic mouse.
4. Characterize the phenotype of the PB-caFGFR1/KDNR bigenic mouse.
5. Generation of a line of the ROSA-caFGFR1 knock-in mice that carry a silent caFGFR1 coding sequence in the ROSA locus for overexpression of the caFGFR1 in target tissues.
6. Generation of a colony of mice carrying the prostate specific disruption of FGFR2 loci for characterization of FGFR2 signaling in the prostate.
7. Generation of a colony of the ARR2PBi-caFGFR1/*Fgfr2*^{c/c} mice for characterization of the receptor specific regulatory function of the FGF signaling axis.

REPORTABLE OUTCOMES

1. Publications:

- a. Jin, C., K. McKeegan, and F. Wang (2003) Transgenic mouse with high Cre recombinase activity in all prostate lobes, seminal vesicle, and ductus deferens. *The Prostate* **57**:160-164.
- b. Jin, C., K. McKeegan, W. Guo, S. Jauma, M. M. Ittmann, B. Foster, N. Greenberg, W. L. McKeegan, and F. Wang (2003) Cooperation between ectopic FGFR1 and depression of FGFR2 signaling in induction of prostatic intraepithelial neoplasia in mouse prostate *Cancer Res.* **63**: 8784-8790.

2. Abstract:

Kerstin McKeegan, Scot Jauma, and Fen Wang (2004) Aberrant FGF signaling axis disrupts homeostasis and induces prostatic intraepithelial neoplasia in the prostate of genetically altered mice. *AACR Annual Meeting Abstract*

3. Presentations:

- a. **The FGF signaling axis in prostate homeostasis and tumorigenesis.** Annual meeting of the Society of Chinese Bioscientists America, Texas Chapter. Houston, TX (2003).
- b. **The FGF signaling axis in prostate homeostasis and tumorigenesis.** Sealy Center for Cancer Cell Biology, University of Texas, Medical Branch. Galveston, TX (2003).
- c. **The FGF signaling axis in prostate homeostasis and tumorigenesis.** Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX (2003)

4. Requests for mouse models through collaborations:

CONCLUSIONS

The prostatic intraepithelial neoplasia (PIN) development and homeostasis perturbation in the prostate of caFGFR1 transgenic mice are caFGFR1 expression level dependent. Repression of the FGFR2 signaling potentiated perturbation of prostate homeostasis induced by the ectopic caFGFR1 kinase. The results further suggest that aberrant FGF signaling in the prostate is a strong factor in disruption of tissue homeostasis, and is a strong promoter for prostate tumor development and progression. In addition, the ARR2PBi-caFGFR1/KDNR mouse model provides a useful tool for evaluating other tumor initiating factors, including genetic instability and other oncogenic lesions in prostate tumorigenesis.

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2. Jin, C., McKeegan, K., Guo, W., Jauma, S., Ittmann, M.M., Foster, B., Greenberg, N.M., McKeegan, W.L., and Wang, F. (2003a). Cooperation between Ectopic FGFR1 and Depression of FGFR2 in Induction of Prostatic Intraepithelial Neoplasia in the Mouse Prostate. *Cancer Res* 63, 8784-8790.
3. Jin, C., McKeegan, K., and Wang, F. (2003b). Transgenic mouse with high Cre recombinase activity in all prostate lobes, seminal vesicle, and ductus deferens. *The Prostate* 57, 160-164.
4. Wang, F., McKeegan, K., Yu, C., Ittmann, M., and McKeegan, W.L. (2004). Chronic activity of ectopic type 1 fibroblast growth factor receptor tyrosine kinase in prostate epithelium results in hyperplasia accompanied by intraepithelial neoplasia. *Prostate* 58, 1-12.

APPENDICES LIST:

1. Jin, C., K. McKeehan, and F. Wang (2003) Transgenic mouse with high Cre recombinase activity in all prostate lobes, seminal vesicle, and ductus deferens. *The Prostate* **57**:160-164.
2. Jin, C., K. McKeehan, W. Guo, S. Jauma, M. M. Ittmann, B. Foster, N. Greenberg, W. L. McKeehan, and F. Wang (2003) Cooperation between ectopic FGFR1 and depression of FGFR2 signaling in induction of prostatic intraepithelial neoplasia in mouse prostate *Cancer Res.* **63**: 8784-8790

Transgenic Mouse With High Cre Recombinase Activity in all Prostate Lobes, Seminal Vesicle, and Ductus Deferens

Chengliu Jin, Kerstin McKeehan, and Fen Wang*

Center for Cancer Biology and Nutrition, Institute of Biosciences and Technology,
Texas A&M University System Health Science Center, Houston, Texas

BACKGROUND. Prostate-specific gene ablation provides a powerful tool for functional characterization of genes that have impact on embryonic development or on other organs, specifically in the prostate. Uniform expression of Cre with high recombinase activity in the prostate is needed for prostate-specific gene ablation based on Cre-loxP recombinations. Currently, available strains of Cre transgenic mice only express Cre recombinase adequately in certain lobes of the prostate. In other lobes, the expression is low and mosaic. Additional strains of transgenic mice expressing high levels of prostate-specific Cre in all prostate lobes would be useful to study the impact of genome manipulation in all prostate lobes.

METHODS. The ARR2PB composite promoter with improved capacity to drive androgen-responsive gene expression was used to initiate expression of a transgene bearing the cDNA encoding a recently modified Cre recombinase with improved recombination activity. In addition, an insulator element from the chicken globin locus that minimized negative effect on transcription of the transgene imposed by chromosome structure was employed. The derived transgenic founders were crossed with the Z/AP reporter mouse and *Fgfr2*^{fl/t} mice bearing loxP flanking the *FGFR2* locus. Immunochemical and mRNA analyses were employed to test expression and efficacy of the Cre recombinase in the prostate and other tissues.

RESULTS. The ARR2PB-Cre transgenic mouse specifically and uniformly expressed Cre recombinase in the dorsal, lateral, ventral, and anterior lobes of the prostate, seminal vesicles, and ductus deferens. The Cre recombinase in these tissues effectively excised loxP flanked DNA fragments in the Z/AP reporter that triggered expression of β -galactosidase, and the loxP-flanked *FGFR2*^{fl/t} locus resulting in specific ablation of *FGFR2* in the prostate.

CONCLUSIONS. Compared with the currently available prostate-specific Cre strains, the new ARR2PB-Cre strain exhibited higher and more uniform expression of Cre recombinase in the prostate as well as in seminal vesicles and ductus deferens. This provides an additional tool for efficient hormone-dependent gene targeting in epithelial cells of all lobes of the adult prostate, seminal vesicle, and ductus deferens. *Prostate* 57: 160–164, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: FGFR; probasin; prostate cancer; prostate-specific gene targeting

INTRODUCTION

Tissue-specific manipulation of embryonic or neonatally lethal target genes or those of widespread consequence in the adult is required for study of the role played in homeostasis and regulation of function of specific adult tissues and organ-specific, age-dependent tumorigenesis. Transgenic mice expressing tissue-specific Cre recombinase crossed with mice bearing loxP flanked genomic DNA containing a target sequence for ablation provides an effective tool for

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*Correspondence to: Fen Wang, Center for Cancer Biology and Nutrition, Institute of Biosciences and Technology, Texas A&M University System Health Science Center, 2121 W. Holcombe Blvd., Houston, TX 77030-3303. E-mail: fwang@ibt.tamushsc.edu
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promoter-controlled and tissue-specific manipulation of the mouse genome. Two strains of transgenic mouse expressing prostate-specific Cre recombinase each constructed with a variant of the androgen-responsive, prostate-specific probasin promoter have been reported [1,2]. The PB-Cre mouse, where expression of the Cre is initiated by the minimal probasin promoter (PB), expresses moderate levels of Cre recombinase in the ventral prostate [2]. The ARR2PB-Cre mouse, where the Cre expression is driven by an improved composite probasin promoter ARR2PB [1,3], appears to express significantly higher levels overall and particularly in the lateral prostate [1]. Recombinase expression in the dorsal and anterior lobes of the prostate appears modest and only apparent in scattered foci reported by a visible Cre/loxP recombination indicator [1].

Two-way directionally specific crosstalk between prostate stroma and epithelium plays an important role in regulating growth, differentiation, and function of the adult prostate. This is thought in part to underpin maintenance of symbiotic homeostasis between the two compartments in both normal prostate and in non-malignant two-compartment tumors. Epithelial cell-specific FGFR2 responding to stromal FGF7/FGF10 has been implicated in the maintenance of epithelial cell homeostasis and the prevention of progression to malignancy [4–7]. Most recently, epithelial cell-derived FGF9 has been proposed as a mediator of back crosstalk to stroma via FGFR3 in a subset of particular stromal cells [20,21].

Loss of epithelial cell FGFR2 and gain of normally stromal FGFR1 in the epithelial cells disturb tissue homeostasis and contribute to malignant progression in transplantable tumor models and are a correlate of some human tumors [5,6,8–13]. We have shown separately that forced ectopic expression and chronic activity of FGFR1 targeted to prostate epithelium with the minimal probasin promoter results in acute prostate hyperplasia accompanied by prostatic intra-epithelial cell neoplasia (PIN) that progresses with age with no signs of overt carcinoma [22].

We predict that the age-dependent chronic activity of ectopic FGFR1 in the epithelium serves as a strong positive promoter of malignant progression in cooperation with loss of restrictive controls such as FGFR2 that promote benign homeostasis. FGFR2 null mice are embryonic lethal [14], and the study of the impact of prostate-specific ablation of the *FGFR2* gene relies on the loxP-Cre recombination system applied to prostate epithelium. Similarly, ablation of FGF9 from the genome causes male-to-female sex reversal, lung hypoplasia, and early postnatal death [15]. Thus, studies of its role as a prostate epithelial cell to stromal signal also require application of a prostate epithelial cell-specific loxP-Cre ablation system.

In preparation for these studies, we report here a third strain of transgenic mouse (ARR2PBi-Cre) expressing Cre recombinase in prostate epithelial cells using the ARR2PB promoter. We utilized an insulator sequence at the 3'-side of the transgene construct to reduce impact on transgene transcription imposed by DNA structure at the insertion site. The strain expressed high levels of Cre recombinase in the epithelium of all prostate lobes, ductus deferens, and seminal vesicles. The Z/AP reporter was homogeneously activated in all lobes of the prostate, seminal vesicles, and ductus deferens in bigenic mice resulting from a cross of the ARR2PBi-Cre mice with Z/AP reporter mice bearing a loxP flanked human placenta alkaline phosphatase (AP) reporter sequence. A cross of the new strain with *FGFR2* loxP mice indicated efficient excision of the *FGFR2* gene. The ARR2PBi-Cre mouse provides an additional tool for high efficiency disruption of target genes in epithelium of prostate, ductus deferens, and seminal vesicles.

MATERIALS AND METHODS

Animals and Reagents

Young adult FVB mice were purchased from Charles River (Wilmington, MA) and young adult Swiss/Webster mice were purchased from Harlan Sprague Dawley (Houston, TX). All oligo-nucleotides were custom ordered from Integrated DNA Technology, Inc. (Coralville, IA). All PCR and luciferase reagents were from Promega (Madison, WI). BM-purple AP substrate was from Roche (Mannheim, Germany).

Construction of the ARR2PBi-Cre Transgene

The ARR2PB composite promoter [3] and Cre cDNA [16] were cloned into SK-PB/SV40t vector that contained a pBluescript SK vector and an RNA splice site and a polyA addition site of SV 40 T-antigens [17]. An insulator element from the 5'-region of the chicken β -globin locus [18] was inserted at the 3'-end of polyA addition site. The ARR2PBi-Cre transgenes were excised with *Bss*III restriction enzyme for pronuclear injection. Sense primer pcre1 (GCCTG-CATTACCGGTCGATGCCAACGA) and antisense primer pcre2 (GTGGCAGATGGCGCGGCAACAC-CATT) were used in PCR screening for Cre cDNA (Fig. 1).

Analyses of Cre Activity

The ARR2PBi-Cre mouse was crossed with the Z/AP transgenic reporter mouse [19] for Cre activity analyses. Sense primer pzap1 (CCCTGCCAAAAT-TATGGGG) and antisense primer pzap2 (ACTATG-GTTGCTGACTAATTG) were used for PCR screening

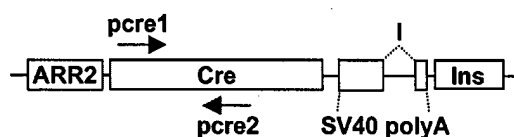


Fig. 1. Construction of ARR2PBi-Cre transgene. Location of the ARR2PB composite promoter (ARR2), Cre cDNA (Cre), an SV40 RNA splice site and polyA addition site (SV40 polyA), and the insulator (Ins) described in the text flanked by the BssHII restriction sites are depicted as boxes. Transgene-specific sense (pcr1) and anti-sense (pcr2) primers used in PCR screening for the transgene are described in Materials and Methods. I, intronic sequence of the SV40 3'-untranslated region.

of human placenta alkaline phosphatase cDNA in Z/AP mouse. The prostate tissues of 6–8 week old bigenic mice (Cr2+/Z/AP+) were excised for alkaline phosphatase activity analysis. Prostates were lightly fixed with 0.2% glutaraldehyde, 0.02% NP-40, and 0.01% sodium deoxycholate in PBS for 4 hr at 4°C; endogenous alkaline phosphatase was heat inactivated by incubation at 70°C for 30 min. The prostate was then incubated with BM-purple (Roche) for 24 hr and whole mounts examined by dissection microscope. For cryosections, prostate tissue sections were prepared and fixed the same as whole mounts of prostate. The tissues were then incubated for 4 hr at 4°C in 10% sucrose in PBS, 4 hr at 4°C in 15% sucrose in PBS solution, and 30 min in 7.5% gelatin in 15% sucrose-PBS. The tissues were then mounted with Tissue-Tek solution (Sakura Finetek, Torrance, CA) and cryosectioned. After heat inactivation of endogenous alkaline phosphatase, the cryosections were stained with BM-purple as described above.

Generation of Prostate-Specific FGFR2 Null Mice

Mice bearing loxP flanked FGFR2 alleles (*Fgfr2^{f/f}*) were gifts from Dr. David Ornitz of the Washington University in St. Louis. Mice carrying the prostate-specific FGFR2 deletion alleles (*Fgfr2^{c/c}*) were generated by crossing the *Fgfr2^{f/f}* mice with the ARR2PBi-Cre mice. Mice carrying *Fgfr2^{f/f}* alleles were identified by PCR with sense primer pFR2-1 (ATAGGACAA-CAGGCGG) and antisense primer pFR2-2 (TGCA-AGAGGCGACCACTCAG). The 500-bp fragment representing the FGFR2 deletion allele was PCR amplified with the same sense primer and antisense primer pAR2 (CATCGCACGGCCAGGTTG), respectively, as suggested by Dr. David Ornitz (personal communication).

RESULTS AND DISCUSSION

We combined three improvements in attempts to generate higher efficiency of the expression of Cre

recombinase in prostate epithelial cells. These included application of the composite rat probasin promoter (ARR2PB) exhibiting two androgen responsive elements (ARE) [3], an improved Cre construct with increased recombination activity [16], and an insulator element from the 5'-region of the chicken β -globin locus at the 3'-end of the transgene polyA addition site [18]. The composite ARR2PB promoter exhibited stronger transcription activity both in vitro and in vivo than other PB constructs, including the minimal probasin promoter [3]. The insulator associates with strong DNase 1 hypersensitive sites and tends to separate chromatin domains with different degrees of condensation, thus, minimizing negative effect on transcription of insulator flanked sequence imposed by chromosome structure [18]. Tandem insertion of multiple copies of the transgene in the genome results in insulator-flanked transgenes that promotes expression of transgenes regardless of insertion location.

The ARR2PBi-Cre transgene (Fig. 1) was injected into pronuclei of fertilized FVB eggs. Transgenic animals resulting from the pronuclear microinjection were identified by PCR analysis and Southern blot hybridization. Two transgenic strains (TG20 and TG22) were generated, both of which stably passed the transgene to offspring. To determine the expression of Cre recombinase, both strains were crossed with the Z/AP double-reporter mouse line [19] that carried a silent human placental alkaline phosphatase gene downstream of a loxP flanked *lacZ* reporter gene. Removal of the *lacZ* sequence by Cre recombinase allows expression of the human alkaline phosphatase reporter gene. The lower urogenital tract of 8-week-old male bigenic mice harboring both the Cre transgene and Z/AP reporter gene was excised and stained with BM-purple alkaline phosphatase substrate for alkaline phosphatase activity. The results demonstrated that one strain exhibited strong staining in all prostate lobes, seminal vesicles, and ductus deferens. No staining was observed in the bladder, urethra, epididymus, and testis (Fig. 2). Separate experiments demonstrated that the Cre recombinase was not expressed in the ampullary gland or the bulbourethral gland of male or the ovaries of female ARR2PBi-Cre mice, nor in any tissue of newborn mice (data not shown). The lower urogenital tract of littermates carrying the Z/AP reporter gene (Fig. 2) or the ARR2PBi-Cre transgene (data not shown) failed to exhibit detectable alkaline phosphatase activity in the examined tissues. This indicated that the Cre transgene was specifically expressed, and the expressed Cre recombinase efficiently excised the loxP-flanked β -geo (LacZ/neoR fusion protein coding sequence) [19] fragment of the Z/AP reporter gene in these tissues. The second strain exhibited strong staining in ventral prostate lobes, but was weak at all other lobes and sites

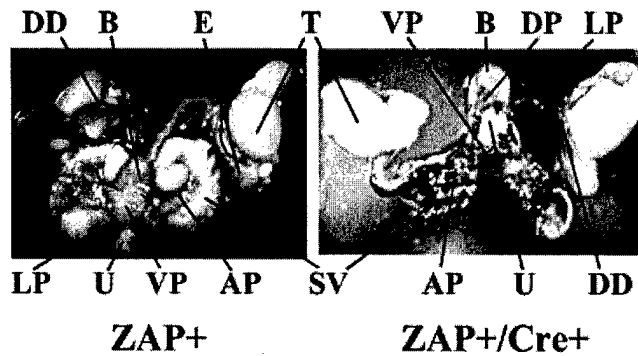


Fig. 2. Alkaline phosphatase activity in urogenital tract whole mounts. The lower urogenital tracts of mice harboring the Z/AP reporter gene only (ZAP+) and both ARR2PB_i-Cre and the Z/AP reporter gene (ZAP+/Cre+) were fixed and the alkaline phosphatase activated by Cre visualized as described in Materials and Methods. The dark blue staining indicates transgenic alkaline phosphatase activity activated due to excision of the floxed β -galactosidase sequence excised from the Z/AP reporter transgene by Cre recombinase. The light blue stain apparent in epididymus was due to endogenous alkaline phosphatase activity remaining after the inactivation procedure. B, bladder; E, epididymus; T, testis; U, urethra; AP, anterior prostate; DD, ductus deferens; DP, dorsal prostate; LP, lateral prostate; SV, seminal vesicle; VP, ventral prostate.

that were positive in the first strain. No staining was observed in the seminal vesicle and ductus deferens.

To determine whether the Cre recombinase was expressed in all epithelial cells of the prostate, the prostates of 8-week-old ARR2PB_i-Cre/Z/AP bigenic males were excised and fixed; cryosections were prepared and then stained with the BM-purple alkaline phosphatase substrate. The ARR2PB_i-Cre1/Z/AP mice exhibited a homogenous staining of epithelial cells of all prostate lobes indicating that the Cre was expressed in most, if not all, prostate epithelial cells (Fig. 3).

We confirmed that the Cre recombinase was expressed in epithelial cells of the ARR2PB_i-Cre mice and efficiently excised the loxP flanked sequences in the *Fgfr2*^{f/f} locus. Genomic DNA from prostates and several other tissues of a 10 weeks old cross between *Fgfr2*^{f/f}/ARR2PB_i-Cre and *Fgfr2*^{f/f} males was extracted and PCR amplified with a pair of primers that only recognized the FGFR2 sequence where the loxP flanked site was removed (*Fgfr2*^{c/c}). Figure 4 indicates that the 500-bp band representing the *Fgfr2*^{c/c} sequence only appeared in the *Fgfr2*^{c/c} prostate. Samples extracted from other tissues including bladder, liver, kidney, and testis from the same mouse, and the prostate from an *Fgfr2*^{f/f} mouse, did not exhibit the characteristic 500 bp band. This further suggested that the Cre recombinase was specifically expressed in the prostate and at levels sufficient to ablate the *loxP*-FGFR2 gene. The impact of

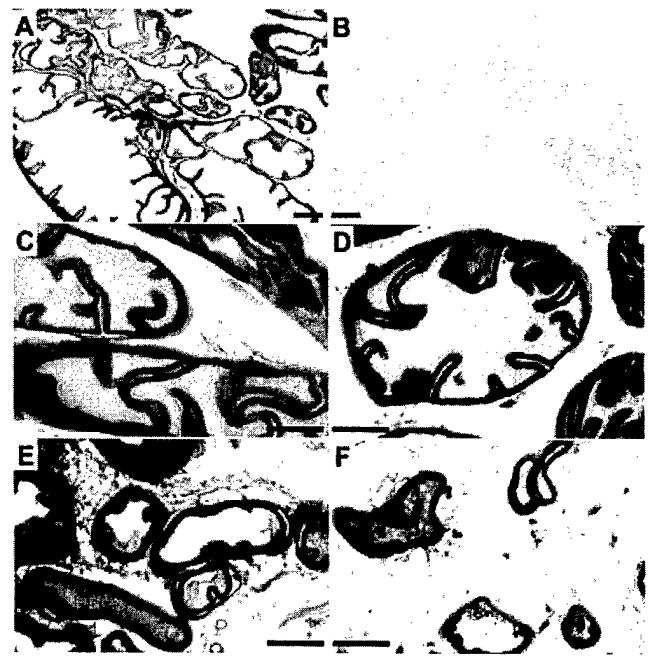


Fig. 3. Epithelial cell-specific alkaline phosphatase in prostates of ARR2PB_i-Cre/Z/AP bigenic mice. Sections of frozen prostate tissues were stained with alkaline phosphatase as described in Materials and Methods. **A, B:** Anterior-dorsal prostate lobes of an ARR2PB_i-Cre-Z/AP bigenic mouse and a Z/AP male as a negative control, respectively, at low magnification; **(C–F)** anterior (C), dorsal (D), lateral (E), and ventral prostate (F) at high magnification. Bar represents 50 μ m.

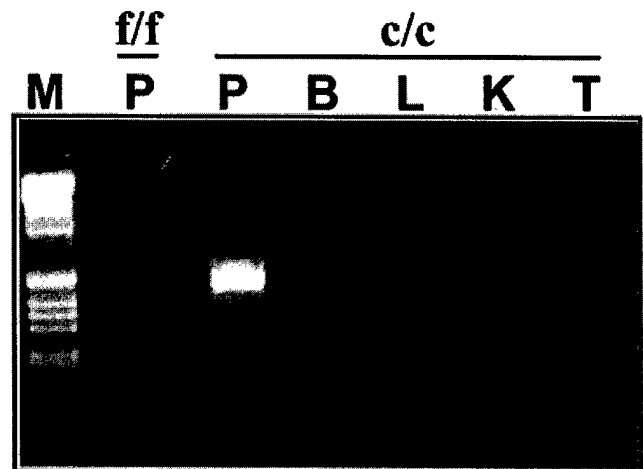


Fig. 4. Excision of floxed *Fgfr2* sequence in *Fgfr2*^{f/f}-ARR2PB_i-Cre bigenic mice. Genomic DNAs from the prostates and the indicated tissues of 10-week-old *Fgfr2*^{f/f} and *Fgfr2*^{f/f}-ARR2PB_i-Cre males were extracted and PCR amplified with a pair of primers specific for the FGFR2 locus across the floxed sequence excised as described in Materials and Methods. The 500-bp fragment represents condition deleted FGFR2 allele was only detectable in *Fgfr2*^{f/f}-ARR2PB_i-Cre bigenic mice. M, 1 kb DNA marker; P, prostate; B, bladder; L, liver; K, kidney; T, testis; f/f, mice bearing the loxP flanked *Fgfr2* loci; c/c, mice bearing prostate-specific disruption of the *Fgfr2* loci.

the conditional ablation of epithelial cell FGFR2 on prostate and compartmental homeostasis during aging in cooperation with other oncogenic lesions, including chronic ectopic epithelial FGFR1, will be the subject of a future report.

In summary, here we reported generation of a Cre transgenic mouse strain (ARR2PB_i-Cre) that specifically expressed Cre recombinase in all prostate lobes, seminal vesicles, and ductus deferens. Compared to the currently available prostate-specific Cre strains (PB-Cre and ARR2PB-Cre), the new ARR2PB_i-Cre exhibited higher and more uniform expression of Cre recombinase in the dorsal, ventral, and anterior prostate lobes. Besides the prostate, the ARR2PB_i-Cre mouse also expressed Cre recombinase in the seminal vesicle and ductus deferens, but not in other tissues outside the urogenital tract. On the one hand, the ARR2PB_i-Cre mouse provides an additional model for high expression of Cre in all prostate lobes where high expression in the seminal vesicle and ductus deferens is tolerable. On the other hand, the mouse may also be useful for hormone-dependent gene targeting in the adult seminal vesicle and ductus deferens.

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Cooperation between Ectopic FGFR1 and Depression of FGFR2 in Induction of Prostatic Intraepithelial Neoplasia in the Mouse Prostate

Chengliu Jin,¹ Kerstin McKeehan,¹ Wei Guo,¹ Scot Jauma,¹ Michael M. Ittmann,² Barbara Foster,⁴ Norman M. Greenberg,³ Wallace L. McKeehan,¹ and Fen Wang¹

¹Center for Cancer Biology and Nutrition, Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston, Texas; ²Departments of Pathology, and ³Cell and Molecular Biology, Baylor College of Medicine, Houston, Texas; and ⁴Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, New York

ABSTRACT

Disruption of the regulatory communication from the stroma to the epithelium mediated by the FGF7/10-FGFR2 signaling axis in the prostate and expression of ectopic FGFR1 in prostatic epithelial cells often correlate with prostate cancer progression both in human and in experimental animals. Ectopic expression of constitutively active FGFR1 mutant (caFGFR1) at low levels in prostate epithelial cells induces low- to intermediate-grade prostatic intraepithelial neoplasia (PIN) within 6–8 months and high-grade PIN in 20–25 months. Depression of the FGFR2 signaling in the prostate also disturbs homeostasis in the prostate and induces prostate hyperplasia. To study whether PIN lesions induced by the caFGFR1 were expression-level dependent, and whether expression of the caFGFR1 and depression of the FGFR2 signaling in the prostate synergistically disturbed prostate homeostasis, we generated two new strains of ARR2PBi-caFGFR1 transgenic mice, which highly expressed caFGFR1 in prostatic epithelial cells. The mice were crossed with KDNr mice to generate ARR2PBi-caFGFR1/KDNr bigenic mice. The ARR2PBi-caFGFR1 mice developed high-grade PIN within 8 months, which was significantly faster than the mice expressing caFGFR1 at low levels. In addition, depression of the FGFR2 signaling clearly promoted perturbation of cellular homeostasis induced by the caFGFR1. The results demonstrated that the PIN development in caFGFR1 transgenic mice was caFGFR1 dosage-dependent, and indicated that the ectopic FGFR1 and the resident FGFR2 in epithelial cells had opposite impacts on intercompartmental homeostasis in the prostate. The bigenic mice provide a model with cooperative aberrations in the fibroblast growth factor signaling axis for evaluation of tumor-initiating events in prostate tumorigenesis.

INTRODUCTION

Prostate cancer is the most frequently diagnosed and the second leading cause of cancer deaths in men in the United States. It is largely unclear how the life-threatening malignant prostate cancer arises as a consequence of changes in relationships between epithelial and stromal compartments cells. These changes allow epithelial cells to escape the homeostatic restraints imposed by the local environment. The fibroblast growth factor (FGF) signaling complex has long been implicated in mediating stromal/epithelial communication and homeostasis, which consists of 23 single polypeptide ligands, 4 transmembrane tyrosine kinase FGF receptors (FGFR), and a pool of highly heterogenic heparan sulfate proteoglycans (HSPGs) in the extracellular matrix (1, 2). Among them, the FGF7/FGF10 and their cognate receptor FGFR2IIIb are partitioned in the stromal and epithelial compartments, respectively, which together with epithelial cell-specific heparan sulfates underlie a directionally specific stromal to

epithelial cell signaling that promotes epithelial cell homeostasis by a net restriction on proliferation and promotion of differentiation (1, 3). Recent studies reveal that stromal cells express FGFR1 and FGFR3 in a cell-specific mode, and that the FGF9 is expressed only in epithelial cells. The FGF9 specifically acts on clonal stromal lines expressing FGFR3, which underlies another directionally specific system within the FGF family from the epithelium to stroma of which the consequences are currently under investigation (4, 5).

Many components of the FGF signaling system are often found aberrantly expressed in prostate tumors (6–8). Particularly, the FGF7/10-FGFR2IIIb and the FGF9-FGFR3 signaling axes, which are common in tissues consisting of epithelial and stroma compartments, are frequently lost during progression to malignancy by splice switching or loss of expression of the gene altogether in human prostate cancers and animal tumor models, including the Dunning and TRAMP tumor models, and other tumor epithelial cells from a variety of parenchymal tissues (4, 9–11). These changes free the premalignant cells from homeostasis-promoting instructive signals elicited by the stroma. In addition to loss of the homeostasis promoting FGF7/10-FGFR2 paracrine signaling axis, fully malignant tumors often ectopically express the FGFR1 kinase that is normally restricted to the stromal compartment, and promotes autonomous growth of tumor cells by establishing an autocrine loop with abnormally expressed autocrine FGFs (8, 10, 12, 13).

Despite high homology in amino acid sequence of the FGFR1 and the FGFR2, especially in the kinase domain, accumulating data indicate that signals elicited by the two FGFRs are different in many tissues, including the prostate (10, 13–16). Depression of the FGFR2 signaling axis by expression of the KDNr, a truncated FGFR2 construct disturbs normal interaction between stromal and epithelial cells, resulting in dysplastic and hyperplastic changes in both compartments, and an increase in neuroendocrine (NE) cell population (17). Forced expression of FGF3 and FGF8 leads to abnormal prostate development and induces prostatic intraepithelial neoplasia (PIN) lesions (18, 19). We reported previously that expression of the constitutively active caFGFR1 mutant at low levels in the prostate-induced prostate hyperplasia accompanied intermediate grade PIN at age 6–8 months and high-grade PIN at age 20–25 months, although no signs of overt carcinoma were observed (20). These suggest that chronic activation of ectopic FGFR1 kinase in prostate epithelial cells upsets homeostasis in the prostate and induces PIN.

To further study the function of FGF signaling in the prostate, we generated two new strains of ARR2PBi-caFGFR1 transgenic mice that highly expressed caFGFR1 in the prostate. The mice were then crossed with KDNr mice to generate ARR2PBi-caFGFR1/KDNr bigenic mice that expressed the ectopic FGFR1 kinase in a background of repressed resident FGFR2 signaling. Histological examination indicated that the ARR2PBi-caFGFR1 mice developed high-grade PIN within 8 months. Disruption of the basement membrane and microinvasion of epithelial foci in space between the basement membrane and the stromal layer were readily seen in the ARR2PBi-caFGFR1 prostate. This indicates that the PIN development and

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homeostasis perturbation in the prostate of caFGFR1 transgenic mice are caFGFR1 expression level dependent. Repression of the FGFR2 signaling potentiated perturbation of prostate homeostasis induced by the ectopic caFGFR1 kinase. Together, these data additionally suggest that aberrant FGF signaling in the prostate is a strong factor in disruption of tissue homeostasis, which, in turn, contributes to prostate tumor development and progression. In addition, the ARR2PB-caFGFR1/KDNR mouse model provides a useful tool for evaluating other tumor-initiating factors including genetic instability and other oncogenic lesions in prostate tumorigenesis.

MATERIALS AND METHODS

Animals and Reagents. Young adult FVB mice (6 weeks old) were purchased from Charles River (Wilmington, MA), and young adult Swiss/Weber mice were from Harlan Sprague Dawley (Houston, TX). The animals were housed in the Institute of Biosciences and Technology Program for Animal Resources facility, and all of the experiments in which animals were involved were performed in compliance with the procedures approved by the Institute of Biosciences and Technology Animal Care Committee. Oligonucleotides were from Integrated DNA Technology, Inc. (Coralville, IA). PCR reagents and restriction enzymes were from Promega (Madison, WI). Pooled mouse monoclonal anti-pan cytokeratin, α -actin, and mouse anti-proliferating cell nuclear antigen (PCNA) antibodies from Sigma-Aldrich (St. Louis, MO). Rabbit antisynaptophysin polyclonal antibody was from Zymed Laboratories, Inc. (San Francisco, CA).

Generation of ARR2PB-caFGFR1 Transgenic Mice. The cDNA encoding for FLAG epitope-tagged caFGFR1 was constructed and inserted in the SSI vector as described (20, 21). A transgene-specific sense primer pFLAG1 and a common FGFR1 antisense primer p240 were used to verify correct insertion and expression of the transgene by PCR (Fig. 1). The transgene was excised and purified for pronuclear microinjection as described (20).

Genomic DNAs were purified from the tails of founder mice at day 7 after birth and screened by PCR. Total RNA was isolated from the mice, and analyzed by reverse transcription-PCR with pFLAG1 and p240 primers. For more quantitative analyses of mRNA by RNase protection assay (RPA), the cDNA template for the 231-bp RNA probe was PCR amplified from human FGFR1 cDNA with forward primer pRPA1 (GCTCTCCCCTCCTCGCAGGAT) and reverse primer pRPA2 (CCTCCAATTCTGTGGTCAGGT), and cloned into the pBluescript SK vector. The RNA probe was transcribed and radiolabeled with α - 32 P]UTP from the template with the Maxiscript kit (Ambion, San Antonio, TX; Ref. 4). The labeled antisense RNA probe was hybridized with 25 μ g of total RNA samples, and the protected fragments

analyzed as described (4). The luciferase coding sequence was excised from the pGL3 vector (Promega) and inserted to the SSI vectors as illustrated in Fig. 1B for construction of the ARR2PB-luciferase reporter.

Histology. The urogenital complex was excised, fixed, and embedded as described (20). One of every five slides was rehydrated and stained with H&E for scanning of general tissue structures of the prostate. Prostatic intraepithelial lesions were classified using the system described recently by Park *et al.* (22), and were additionally accessed by a pathologist (M. M. I.). Immunohistochemistry analyses with anti-pancytokeratin, anti- α -actin, anti-PCNA, and anti-synaptophysin antibodies were performed on 5- μ m paraffin sections. All of the samples, excluding those for anti-synaptophysin staining, were incubated at 95°C in 10 mM citric acid buffer (pH 6.0) for 20 min for antigen retrieval. No antigen retrieval was carried out for anti-synaptophysin staining per the manufacturer's suggestion. The sections were then incubated with 5% BSA for 30 min, followed by primary antibodies (1:200) for 1 h. The ExtrAvidin Peroxidase Staining kit (Sigma-Aldrich) was used as an amplification system according to the manufacturer's instructions where indicated. Otherwise, the specific bound primary antibodies were detected and visualized with alkaline phosphatase-conjugated secondary antibodies.

For periodic acid Schiff's staining, the sections were rehydrated and treated with 0.5% periodic acid at 37°C for 30 min. After being rinsed with water, the slides were stained with Schiff's reagent (Sigma Co.) for 15 min and counterstained with hematoxylin according to the manufacturer's instructions.

Luciferase Assay. The ARR2PB-luciferase report construct was transiently introduced to DTE-AR prostate epithelial cells that are stably transfected with androgen receptor. Four h after the transfection, the cells were harvested and replated in a 24-well plate in the medium containing 5% charcoal-stripped fetal bovine serum in the presence or absence of 5 mM 5 α -dihydrotestosterone as indicated. The cells were lysed with the lysis buffer (Promega) after incubation at 37°C overnight; the cell lysates were mixed with 25 μ l of luciferase substrate, and the fluorescence light intensity was measured with a microplate scintillation counter (Packard, Meriden, CT).

RESULTS

Generation of ARR2PB-caFGFR1 Mice. We previously generated PB-caFGFR1 transgenic mice that used the minimal probasin promoter (PB) to target expression of the caFGFR1 specifically to prostate epithelial cells. Ectopic presence and chronic activation of the FGFR1 in mouse prostate epithelial cells, although at low levels, induced age-dependent progressive PIN accompanied by stroma thickening (20). To determine whether expression of the caFGFR1 kinase at high levels induced more profound phenotypes in younger mice, we constructed a new transgene ARR2PB-caFGFR1 (Fig. 1A) with the improved ARR2PB composite promoter to direct expression of the transgene in prostate epithelium. *In vitro* data clearly demonstrated that the ARR2PB promoter exhibited a strong androgen-dependent activity in initiating transcription of the ARR2PB-luciferase reporter (Fig. 1B) in DTE epithelial cells derived from the Dunning 3327 prostate tumor (Fig. 1C). As in the PB-caFGFR1 transgene, an artificial FLAG epitope was tagged to the NH₂ terminus of FGFR1 to facilitate identification of the transgene products. The ARR2PB-caFGFR1 transgene was excised for generation of transgenic mice in FVB inbred background. Two ARR2PB-caFGFR1 transgenic lines were established, both of which stably passed the transgenes to offspring.

Expression of the ARR2PB-caFGFR1 Transgene in the Prostate. To assess expression of caFGFR1 in the prostate, both ARR2PB-caFGFR1 transgenic mice and wild-type (WT) littermates of 6–8 weeks were sacrificed, and total RNAs were extracted from the prostates. Expression of caFGFR1 was evaluated with reverse transcription-PCR with the primers showed in Fig. 1A. The results clearly showed that the ARR2PB-caFGFR1 was expressed only in the prostate of transgenic mice. In addition, the 350-bp fragment representing caFGFR1 was not present in samples without reverse transcription, indicating that the fragment was amplified from the first

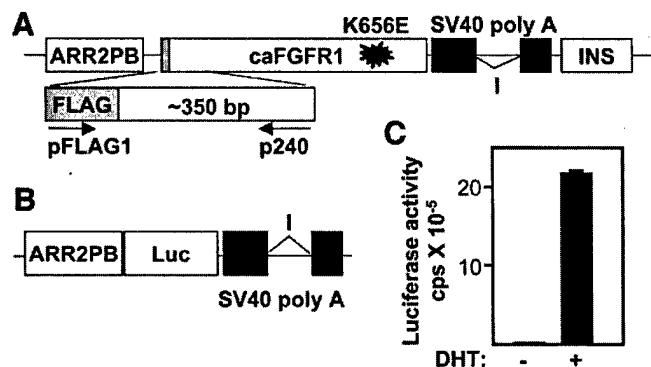


Fig. 1. Construction the ARR2PB-caFGFR1 transgene. A, schematic of the caFGFR1 transgene. The human two immunoglobulin-loop isoform of FGFR1 bearing the K656E mutation and an artificial FLAG epitope was inserted downstream of the ARR2PB composite promoter. The arrows indicate transgene specific primer pFLAG1 and p240 used in PCR screening and reverse transcription-PCR analysis. B, schematic of the ARR2PB-luciferase reporter gene. C, androgen-dependent expression of the ARR2PB-luc reporter. The DTE-AR cells were transiently transfected with the ARR2PB-luc reporter DNA and cultured in the presence or absence of DHT. The expressed luciferase was assessed. Data were means of duplicated samples. Luc, luciferase; ARR2PB, the composite probasin promoter; SV40 polyA, SV40 splice site and polyA addition site; I, intronic sequence of SV40 T antigens; INS, insulator.

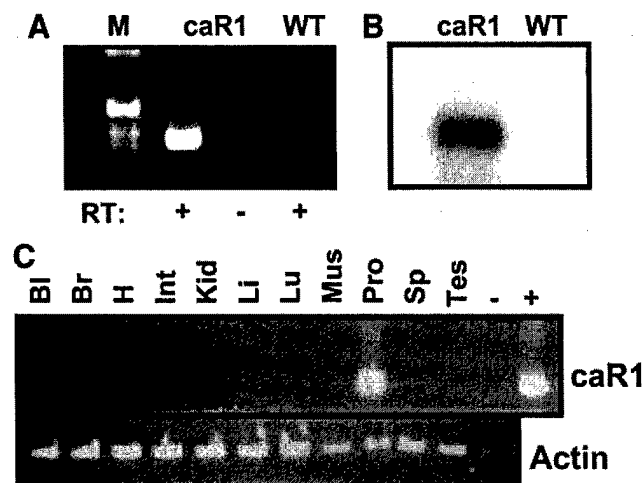


Fig. 2. Expression of the caFGFR1 in the prostate. Total RNA samples purified from the indicated tissues were analyzed with reverse transcription-PCR (A and C) or RPA (B). The 230-bp actin fragments in C were amplified from the same first-strand cDNAs as loading controls. *Bl*, bladder; *Br*, brain; *Int*, small intestine; *H*, heart; *Kid*, kidney; *Li*, liver; *Lu*, lung; *M*, molecular weight ladders; *Mus*, skeletal muscle; *Pro*, prostate; *Sp*, spleen; *Tes*, testis; -, negative control without the first-strand cDNA; +, positive control with FGFR1 cDNA; RT, reverse transcription.

strand cDNAs specifically (Fig. 2A). Furthermore, RPA results demonstrated that the caFGFR1 was expressed strongly in the prostate of ARR2PBi-caFGFR1 transgenic mice (Fig. 2B). Under similar conditions, expression of caFGFR1 was undetectable in the prostate of PB-caFGFR1 mice with RPA (data not shown), and the 350-bp fragment representing caFGFR1 can only be weakly detected in oligodeoxythymidylic acid column purified mRNA samples from the PB-caFGFR1 prostate (20), which suggests that expression of caFGFR1 was significantly improved in the prostate of ARR2PBi-caFGFR1 mice. To additionally determine whether the ARR2PBi-caFGFR1 transgene was expressed only in the prostate, total RNA samples were extracted from different tissues including bladder, brain, heart, intestine, kidney, liver, lung, muscle, spleen, prostate, and testis. PCR analysis clearly indicated the 350-bp fragment was only amplified from the RNA extracted from the prostate (Fig. 2C). The 230-bp actin fragments were amplified from the same first-strand cDNA pools for loading controls as described (4).

ARR2PBi-caFGFR1 Mice Developed High-Grade PIN within 8 Months. In general, ARR2PBi-caFGFR1 mice exhibited no significant phenotype and were reproductively active before the age of 3–4 months, which were similar to PB-caFGFR1 mice that expressed caFGFR1 kinase at low levels in the prostate. ARR2PBi-caFGFR1 males frequently lost reproductive ability after the mice had reached the age of 6 months. The average wet tissue weight of the ARR2PBi-caFGFR1 prostate was 131 ± 20 mg ($n = 13$), and the average prostate tissue weight of WT littermates was 78 ± 14 mg ($n = 30$). Clearly, the prostate of ARR2PBi-caFGFR1 mice was significantly larger than that of WT littermates ($t < 0.001$). To study the time-dependent developments of prostatic lesions induced by the caFGFR1 kinase at high dosages, prostate tissues were collected from ARR2PBi-caFGFR1 mice and WT littermates at 3–12 months of age. The prostate tissues were completely sectioned; one of every five slides was selected, stained with H&E, and examined histologically. No significant difference between the prostate tissues of ARR2PBi-caFGFR1 mice and WT littermates at the age of 3–4 months was observed (data not shown). However, most ARR2PBi-caFGFR1 prostates developed PIN 3 to PIN 4 lesions in every lobe when the mice were older than 8 months (Table 1). In the foci with high-grade PIN, the lumens were filled with disorganized, atypical epithelial cells

(Fig. 3). The nuclear to cytoplasmic ratio was significantly increased. Hyperchromatic nuclei with clearly visible nucleoli were present. Areas of cells with a cribriform pattern were observed in both dorsal-lateral and anterior lobes. Frequently, epithelial cells were observed pushing into the stromal layers and formed microinvasion foci in the stromal layer. Mitotic figures were also apparent in the foci with high-grade PIN. Cellular and nuclear atypia were clearly more pronounced compared with that of PB-caFGFR1 prostates that only expressed the caFGFR1 at low-levels and developed intermediate PIN at similar ages.

Table 1 Pathology of ARR2PBi-caFGFR1 mice

Age (mo)	Tissue no	Line no ^a	PIN ^b Lesions			Epithelial cell push through	Cribriform structures
			AP	DLP	VP		
6	373	31	3	3	3	Yes	Yes
7	311	31	3	3	2	Yes	Yes
7	314	31	3	3	2	Yes	No
7	315	31	3	3	2	No	No
8	289	21	4	4	4	Yes	Yes
9	250	21	3	3	4	Yes	Yes
10	370	21	3	4	4	Yes	Yes
10	371	31	4	4	4	Yes	Yes
11	365	21	4	4	3	Yes	Yes
11	367	21	3	3	2	No	No
11	466	21	3	3	4	Yes	Yes
12	430	21	3	4	4	Yes	Yes
12	433	21	4	3	3	Yes	Yes
15	426	21	4	4	3	Yes	Yes

^a Line 21 and 31 are independent ARR2PBi-caFGFR1 transgenic lines generated through microinjections.

^b PIN, prostatic intraepithelial neoplasia; AP, anterior prostate; DLP, dorsal lateral prostate; VP, ventral prostate.

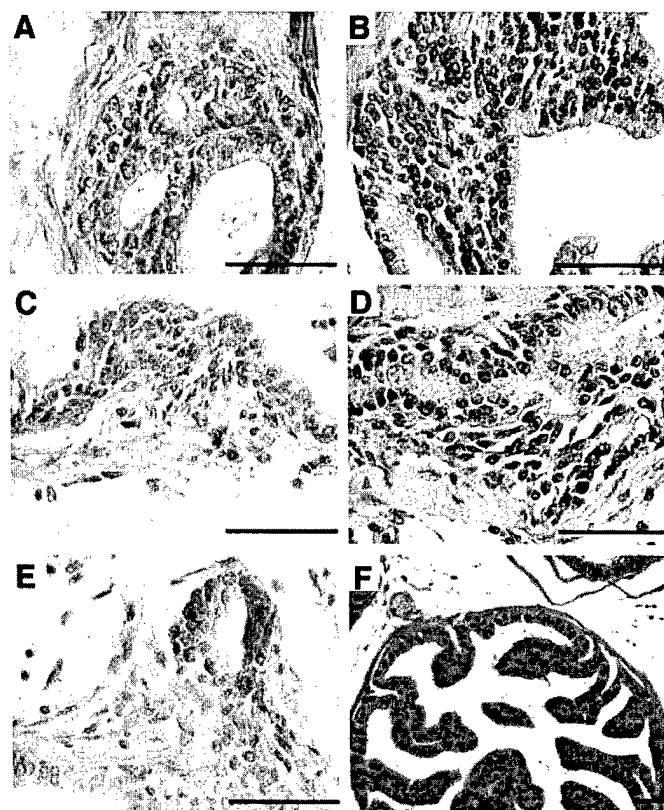


Fig. 3. High-grade PIN in the prostate of ARR2PBi-caFGFR1 transgenic mice. Sections were prepared from prostates of 30-week-old ARR2PBi-caFGFR1 transgenic mice (A–E) and WT FVB mouse (F) as described in “Materials and Methods,” and representative fields are shown. Note the cribriform structures are illustrated in A; hyperplasia of epithelial cells in B; discontinued stromal-epithelial boundaries in C and D; and bulging of epithelial cells into the stroma is shown in E. Bar = 50 μ m.

Fig. 4. Immunohistochemical analysis of cytokeratins, α -actin, and PCNA. Sections from the prostates of 8–10 months old mice were stained with anti-pan-cytokeratin (A–C), anti- α -actin (D–F), and anti-PCNA (G–I) as described in "Materials and Methods." Typical positive staining of the PCNA was indicated with arrows. A, B, D, E, G, and H, prostate sections of ARR2PBi-caFGFR1 mice; C, F, and I, WT FVB mice.



Histochemical Characterization of the Prostate of ARR2PBi-caFGFR1 Mice. To determine whether cells within the PIN foci in the ARR2PBi-caFGFR1 prostate expressed cytokeratins that are characteristic markers of well-differentiated prostate epithelial cells, the prostate sections of the ARR2PBi-caFGFR1 and WT littermates were stained with anti-pancytokeratins as described (20). The results clearly revealed that the expression of cytokeratins was reduced significantly in epithelial foci with high-grade PIN lesions, especially those with cribriform structures. It was necessary to extend the developing time to demonstrate some faint staining in these slides, which increased the background staining (Fig. 4A), whereas the cells in relatively normal areas, even those adjacent to the PIN foci, still highly expressed cytokeratins (Fig. 4B) as the cells in WT prostates (Fig. 4C). In addition, the high-grade PIN foci, particularly those with cribriform structures, often had a disrupted stromal layer indicated by discontinuous and faint staining of smooth muscle cell characteristic α -actin (Fig. 4, D and E). Similarly, it was necessary to extend the development time for these slides to demonstrate weak staining of α -actin, which was almost indistinguishable from the background staining. In contrast, the epithelial compartment of WT prostates was usually surrounded by well-organized stromal cells readily to be demonstrated with α -actin staining (Fig. 4F). To determine whether the cells in the ARR2PBi-caFGFR1 prostate were actively engaged in proliferation, the prostate sections from both ARR2PBi-caFGFR1 and WT littermates were stained with anti-PCNA antibody. The results indicated that the PCNA was readily detected in the ARR2PBi-caFGFR1 prostate (Fig. 4, G and H) but not in WT prostate (Fig. 4I). Periodic acid Schiff's staining, which stained polysaccharides in the basement membrane that separated the epithelial and stromal compartments, revealed that the basement membrane in the ARR2PBi-caFGFR1 prostate was discontinuous, especially around the high-grade PIN foci (Fig. 5, A–C). Frequently, the overproliferating epithelial cells disrupted the basement membrane, invaded to the stromal compartment, and formed small epithelial foci in the space between the basement

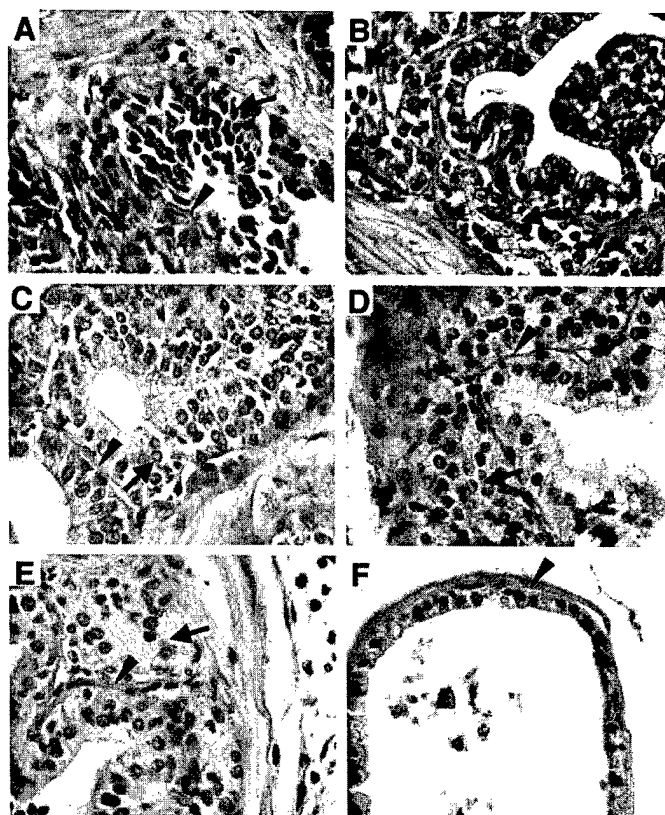


Fig. 5. Disruption of the basement membrane in high-grade PIN foci. Sections from the ARR2PBi-caFGFR1 (A–C), PB-caFGFR1/KDNR bigenic (D and E), and WT (F) prostate were stained with periodic acid Schiff's and counterstained with hematoxylin. Note the thin pink-stained basement membrane indicated with arrowheads separating the epithelial-stromal compartmentalization was often disrupted in the ARR2PBi-caFGFR1 and the PB-caFGFR1/KDNR prostate. Arrows indicated microinvasion of dysplastic epithelial cells into the underlying stroma.

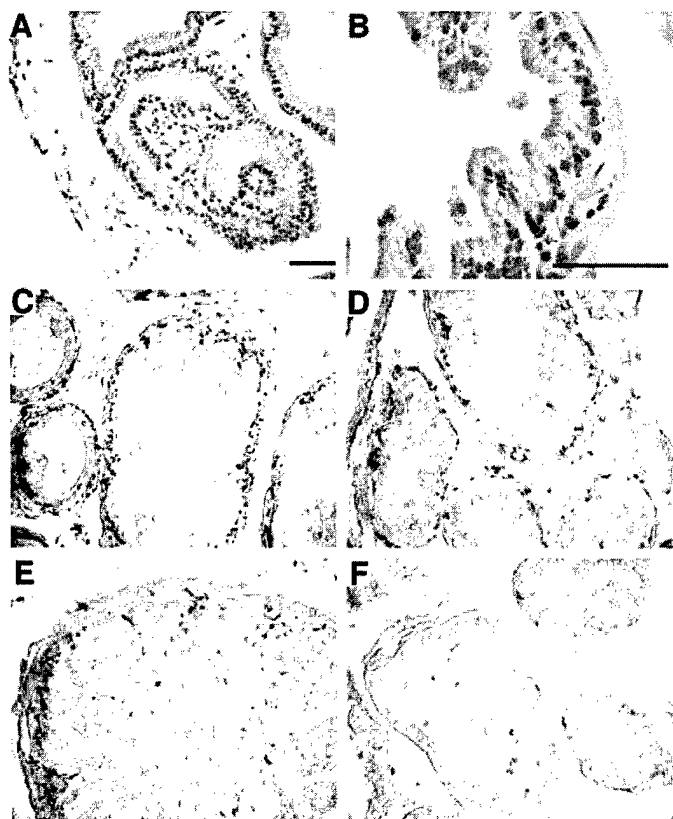


Fig. 6. The increased NE cell population in the prostate of ARR2PBi-caFGFR1/KDNR bigenic mice. (A and B), sections from the ARR2PBi-caFGFR1 prostate were H&E stained for histological characterization. Sections from the prostate of the ARR2PBi-caFGFR1/KDNR (C), KDNR (D), ARR2PBi-caFGFR1 (E), and WT mice (F) were stained with antisynaptophysin antibody as described in "Materials and Methods." Bar in A and B represents 50 μ m.

membrane and stromal cells as indicated with arrows in Fig. 5, A–C. This indicated that the epithelial cells in these foci were not just pushed through the stromal layer and formed glandule-like structures. Instead, these epithelial cells formed microinvasion foci in the stromal compartment, which were more advanced lesions than high-grade PIN.

Cooperation between Ectopic Expression of FGFR1 and Depression of FGFR2 Signaling in Perturbation of Prostate Homeostasis. Both *in vivo* and *in vitro* analyses of the Dunning and the TRAMP tumor models reveal that FGFR2 promotes homeostasis in the prostate (9, 13, 14, 23). Expression of the KDNR that inhibits endogenous FGFR2 kinase through dimerization in prostate epithelial cells disrupts association between epithelial and stromal cells, causes prostate hyperplasia, and increases the NE cell population in the prostate (17). To determine whether disruption of endogenous FGFR2 signaling axis and ectopic appearance of FGFR1 in prostate epithelial cells had synergistic effects in disturbing prostatic homeostasis, we generated ARR2PBi-caFGFR1/KDNR bigenic mice by crossing. Like the parental strains, young adult bigenic mice did not exhibit significant abnormality in the prostate. Yet, the bigenic prostate gradually developed high-grade PIN foci when the mice were older than 6 months (Fig. 6, A and B), which was similar to the mice only expressing high levels of caFGFR1. To determine whether ectopically expressed FGFR1 kinase additionally increased the NE cell population, tissue sections of prostates from the ARR2PBi-caFGFR1/KDNR, ARR2PBi-caFGFR1, KDNR, and WT mice were immunohistochemically stained with anti-synaptophysin antibody. The results clearly demonstrated that the NE cell population in the bigenic mice

(Fig. 6C) was significantly higher than in both KDNR (Fig. 6D) and ARR2PBi-caFGFR1 (Fig. 6E) counterparts. As reported earlier (17), the KDNR prostate had an increased NE cell population in the prostate (Fig. 6D). In addition, the NE cell population in the ARR2PBi-caFGFR1 prostate was also somewhat increased (Fig. 6E) compared with that of WT littermates (Fig. 6F).

To determine whether repression of FGFR2 signaling promoted PIN lesion development in PB-caFGFR1 mice that only expressed caFGFR1 kinase at low levels in the prostate, we then generated PB-caFGFR1/KDNR bigenic mice by crossing. The prostate tissues of the bigenic and both monogenic parental strains were collected for pathological examination at different ages. The PB-caFGFR1 and KDNR parental strains usually developed high-grade PIN foci only when the mice were older than 18 months (20). In contrast, the prostate of PB-caFGFR1/KDNR bigenic mice frequently developed high-grade PIN within 8 months (Fig. 7, A–D), which was significantly earlier than both parental strains. Most of the lumen of the gland was filled with multiple layers of atypical epithelial cells with hyperchromatic nuclei and prominent nucleoli. Cellular and nuclear atypia was clearly more prominent compared with the intermediate PIN in the PB-caFGFR1 and the KDNR mice at similar ages (17, 20). The nuclear to cytoplasmic ratio was significantly increased in the cells within the PIN foci as well (Fig. 7, B–D). Furthermore, the NE cell population in the PB-caFGFR1/KDNR bigenic prostate also increased significantly (Fig. 7F). Periodic acid Schiff's staining indicated disruption of basement membrane and microinvasion of epithelial foci in the stromal compartment (Fig. 5, D–E). Trichrome staining also revealed that hyperplastic stroma did not exhibit excessive collagen staining in either ARR2PBi-caFGFR1/KDNR or PB-caFGFR1/KDNR bigenic mice (data not shown), as reported earlier (17).

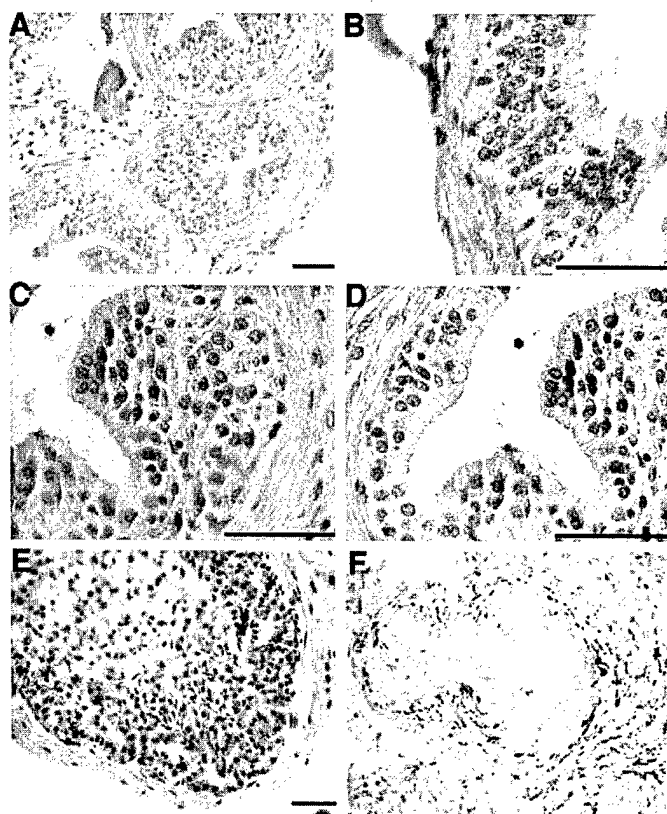


Fig. 7. Disruption of the FGFR2 signaling axis promoted development of PIN induced by the ectopic FGFR1 kinase in prostate epithelial cells. Sections from the prostates of 8-month-old ARR2PBi-caFGFR1/KDNR bigenic (A–D) and KDNR (E) mice were stained with H&E as described. F, anti-synaptophysin immunohistochemical staining of the same bigenic prostate tissue section as described in Fig. 6.

DISCUSSION

Expression and activation of the FGF signaling axis are highly spatial and temporal specific. We reported previously that ectopic expression of low levels of constitutively active FGFR1 mutant in prostate epithelial cells perturbed homeostasis in the prostate and induced development of PIN in the prostate over time (20). Here we additionally demonstrated that an increase in expression of the caFGFR1 kinase dramatically accelerated development of PIN in the prostate. Most ARR2PBi-caFGFR1 transgenic mice developed high-grade PIN within 8 months. Foci with cribriform structures in lumen of the prostatic acini and epithelial cells pushing through stromal compartments were readily seen throughout the prostate. These features are consistent with recently defined PIN 4 (22), which is considered the highest grade of PIN preceding development of overt carcinoma. Epithelial cells in most high-grade PIN foci failed to express cytokeratins that were characteristically expressed in highly differentiated epithelial cells. Furthermore, the stromal cells surrounding the epithelial cells in high-grade PIN foci also failed to express α -actin, a characteristic marker associated with differentiated smooth muscle cells or lineages related to them. The basement membrane surrounding the epithelial compartment was often disrupted, especially in the foci with high-grade PIN. These results indicated that development of PIN induced by the ectopic caFGFR1 kinase was positively correlated with the expression level. Secondly, depression of the FGFR2 signaling axis by expression of KDNr, a dominant kinase inactive construct of FGFR2, in the same prostate epithelial cells contributed to development of PIN induced by low levels of caFGFR1 kinase. Expression of KDNr did not significantly accelerate PIN development induced by high-level caFGFR1 kinase, but expression of caFGFR1 and depression of FGFR2 synergistically increased the population of NE cells in the prostate. The results additionally demonstrated that on the one hand the FGF signaling axis was important in regulating homeostasis in the prostate. On the other hand, aberrant FGF signaling in the prostate perturbed homeostasis in the prostate and induced PIN lesions.

Normally, it takes years to decades for development of full-blown, life-threatening malignant prostate cancers from dormant prostate tumor cells. It is believed that prostate cancers arise from the epithelial cells with progressive and collective changes that allow them to escape homeostatic restraints imposed by the local environment. Escape from the FGF7/10-FGFR2 paracrine regulation from stroma to epithelium, which has a net effect of homeostasis and promotion of differentiation, and expression of ectopic FGFR1 kinase that promotes autonomous growth of tumor cells are the hallmark changes in progression to malignancy of tumors originating from two-compartment tissues, including human prostates, and from experimental animal models (1). Yet, no overt tumor was found in the mice with the aberrant FGF signaling axis even in prostates that developed high-grade PIN and foci indicative of microinvasion rapidly after maturation. This may be due to the androgen dependence and the dependence of high activity of the PBARR2 composite promoter on highly differentiated prostate epithelial cells. Once the caFGFR1-expressing cells dedifferentiate or relax dependence on androgen passed certain stages during the progression to malignancy, potentially expression of the ARR2PBi-caFGFR1 transgene is diminished as the T antigens in the TRAMP mice (23). These cells may be frozen at the particular stage of malignancy, revert to relatively "normal" stages, or enter apoptosis due to lack of sustained high levels of the supporting ectopic FGFR1 kinase. Currently, we are establishing new lines of mice that constantly express caFGFR1 specifically in the prostate independent of androgen and differentiated state. These mice may provide new information on whether overexpression of the ectopic FGFR1 kinase

alone or in cooperation with other lesions as FGFR2 depression will induce fully malignant tumors in the prostate.

Changes in HSPG in the extracellular matrix may also be important for ectopic FGFR1 kinase to abrogate growth restraints on prostate epithelial cells imposed through interaction with the stromal compartment and to induce prostatic lesions beyond PIN. Accumulating data indicate that HSPGs contribute to the FGF signaling axis, possibly through the following mechanisms: (a) interaction with HSPG restrains unliganded FGFR dimers in an inactive conformation that is abrogated by docking of the FGF to ligand-binding pockets, and confines ligand-specificity of the binary complex (2, 24–29); and (b) intracellular domain of HSPG core proteins may be involved in organizing downstream substrate complexes around the FGFR kinase (25, 30). Hence, changes in structure of heparan sulfate side chains and core proteins of HSPGs may contribute to the signaling specificity of FGFR kinases in the prostate. Although the four FGFRs share high homology in amino acid sequence and form heterodimers in the absence of heparan sulfate cofactors (31), FGFR1 and FGFR2 appear to be able to elicit receptor-specific regulatory activities when expressed in mammalian host cells, including prostate epithelial cells, suggesting no formation of FGFR1/FGFR2 heterodimers on the surface of these cells where FGFR-specific HSPGs are present (10, 14, 15). Hence, it is very unlikely that products of the caFGFR1 and KDNr transgenes would form heterodimers in epithelial cells of the transgenic prostate, because FGFR2-specific HSPGs are highly abundant in the extracellular matrix of prostate epithelial cells (25). It is conceivable that the two transgenes synergistically perturb prostate homeostasis through independent mechanisms.

The NE cells, which are present in normal, hyperplastic, and dysplastic prostate tissues, and are located in all regions of the human prostate, represent a minor epithelial cell population. The function of NE cells in the prostate, as well as the clinical significance of the NE cell in prostate cancer, is unclear, although several studies reveal an increase of NE cell population in high Gleason score prostate lesions. Currently, it is believed that NE cells may be important in regulating the growth, differentiation, and function of the prostate through secreting autocrine and paracrine peptides (23, 32). Normally, NE cells in the prostate show no proliferative activity, do not express androgen receptor, and are not androgen dependent (33). Hence, it is more likely that expression of the ARR2PBi-caFGFR1 and KDNr transgenes in prostate epithelial cells induces paracrine factors that, in turn, induce overproliferation of the NE compartment in the prostate. It should be noted that the appearance of a significantly elevated NE cell population in the ARR2PBi-caFGFR1 and KDNr transgenic mice differed from the situation observed in the TRAMP model, in which advanced prostate cancers were observed to take on a NE phenotype as a stochastic event related to progression in this animal model system (23). Elevation of the NE cells in the ARR2PBi-caFGFR1 and KDNr transgenics resulted from the selective expansion of the non-NE epithelial cells of the prostate, as a function of the deregulated FGF signaling axis, is similar to stromal population changes in rat prostate tumor (Dunning R3327PAP) as a function of changes in the FGF signaling axis (4, 5). Although the NE cells in WT prostate were mainly restricted to the basement membrane, both caFGFR1/KDNr bigenic and KDNr prostate showed a significant amount of NE cells in the hyperplastic stromal layer (Fig. 7F). These data additionally suggest that an aberrant FGF signaling axis in prostate epithelial cells induces abnormal expression of regulatory paracrine factors that directly or indirectly disturb homeostasis in the prostate to increase the population of NE and alter the stromal cell population in the prostate.

The KDNr was targeted for expression in prostate epithelial cells with the minimal PB promoter (17). Due to relatively weak transcription initiation activity of the promoter, expression of the KDNr in

prostate epithelial cells was not high. Probably only a fraction of resident FGFR2 kinase in KDNr prostate epithelial cells is suppressed because the degree of dominant-negative inhibition through heterodimerization requires high levels of the dominant species. It is not surprising that expression of the KDNr alone only induces a mild phenotype in the prostate as reported (17). Yet, even at low levels, the kinase inactive KDNr and ectopic FGFR1 kinase cooperatively perturbed homeostasis in the prostate and induced advanced PIN. This additionally demonstrated the homeostasis-promoting role of FGFR2 in the prostate, and that FGFR1 and FGFR2 elicit different receptor-specific regulatory activity in the prostate. It is conceivable that full disruption of the FGFR2 signaling axis in the prostate may induce a more severe perturbation of prostate homeostasis, and this is under investigation. Furthermore, expression of high-level caFGFR1 in a background completely deficient in FGFR2 will rule out the possibility that caFGFR1 perturbs FGFR2-mediated homeostasis in the prostate through heterodimerization and inhibition of FGFR2 signaling in the prostate.

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